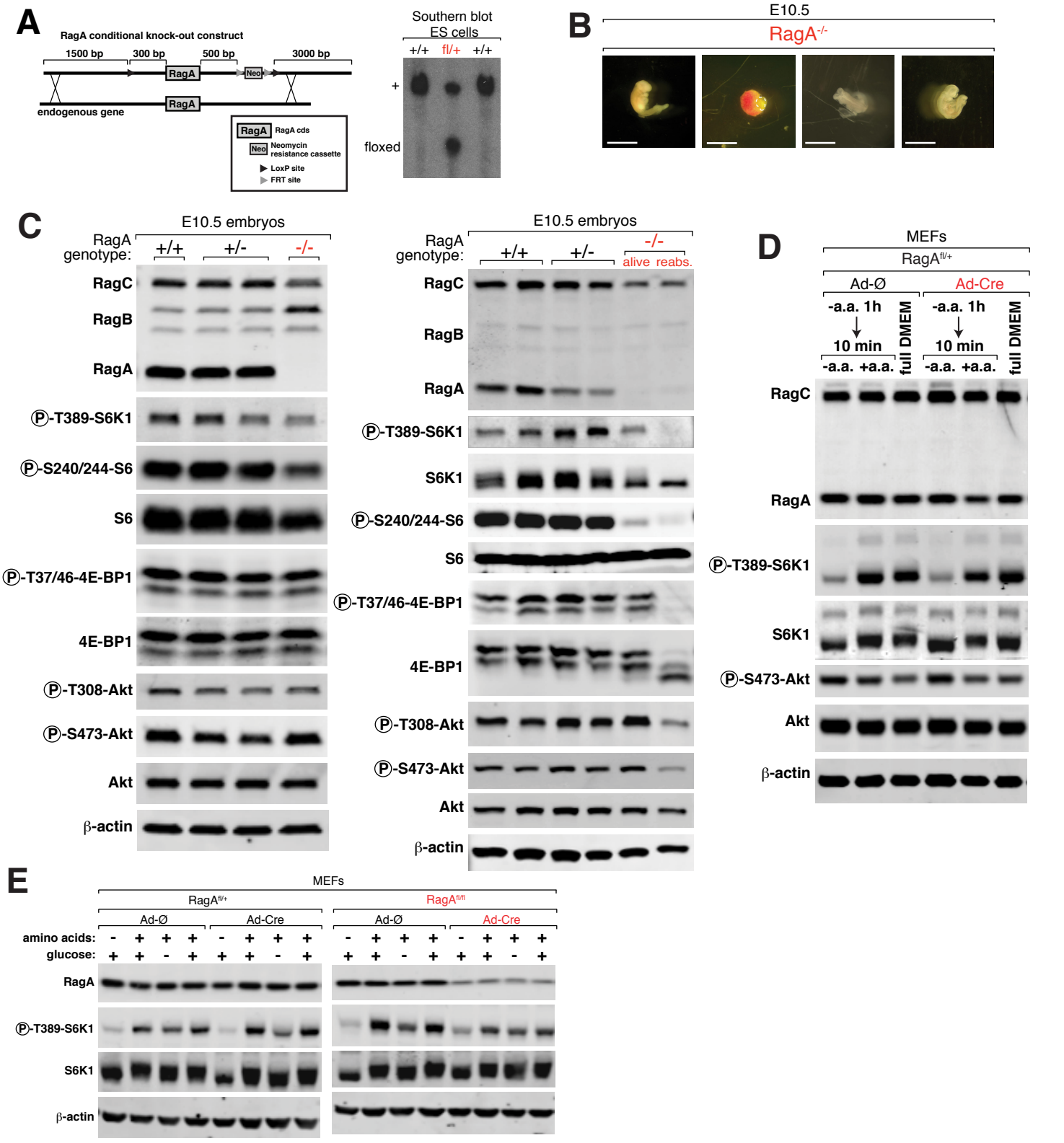
Supplementary Information:

-Supplementary Figures 1, 2, 3 and 4, related to Main Figures 1, 2 3, and 4, respectively.

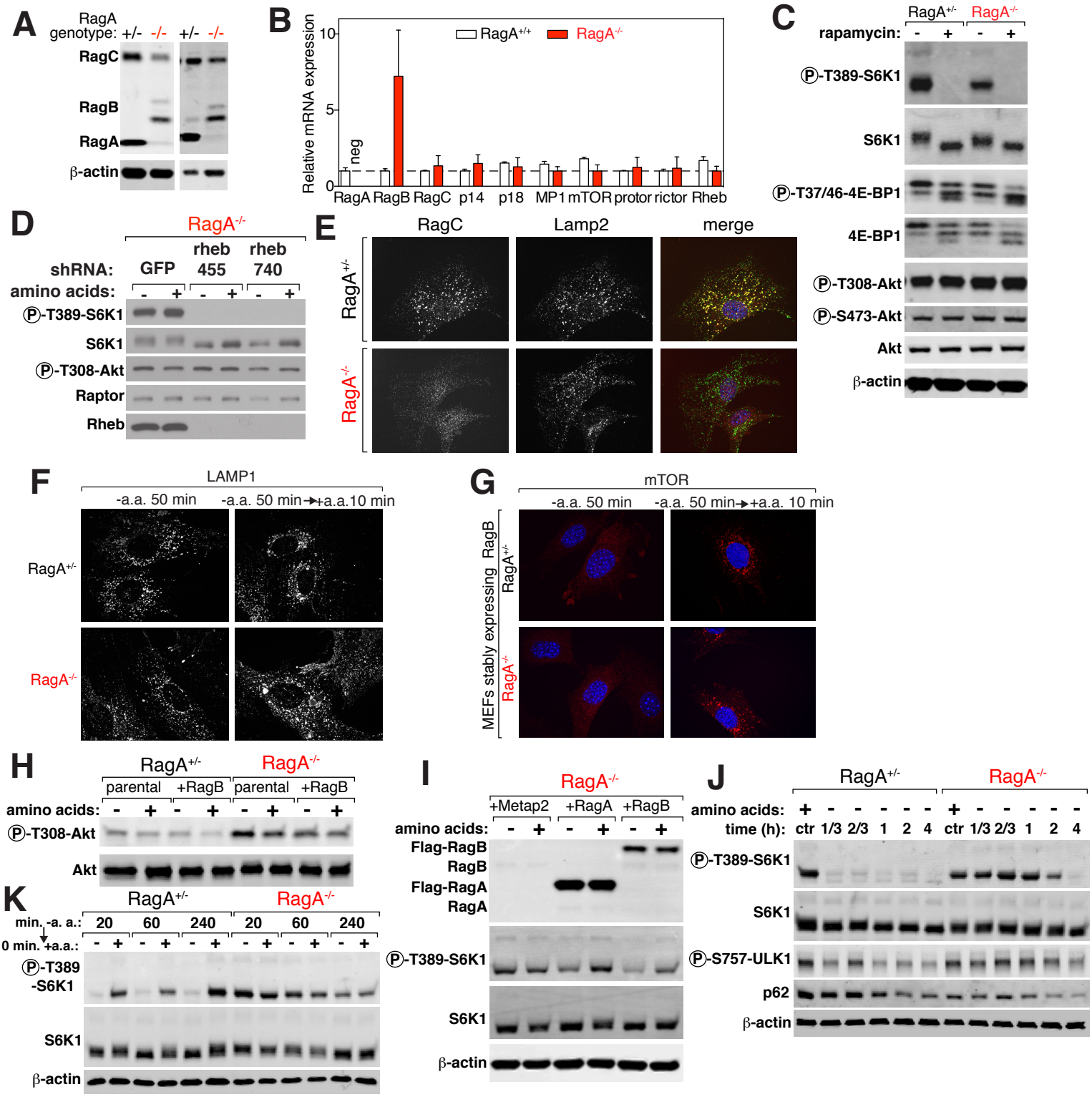
-Supplementary Table 1, related to Figure 1

-Supplementary Table 2, related to Figure 3

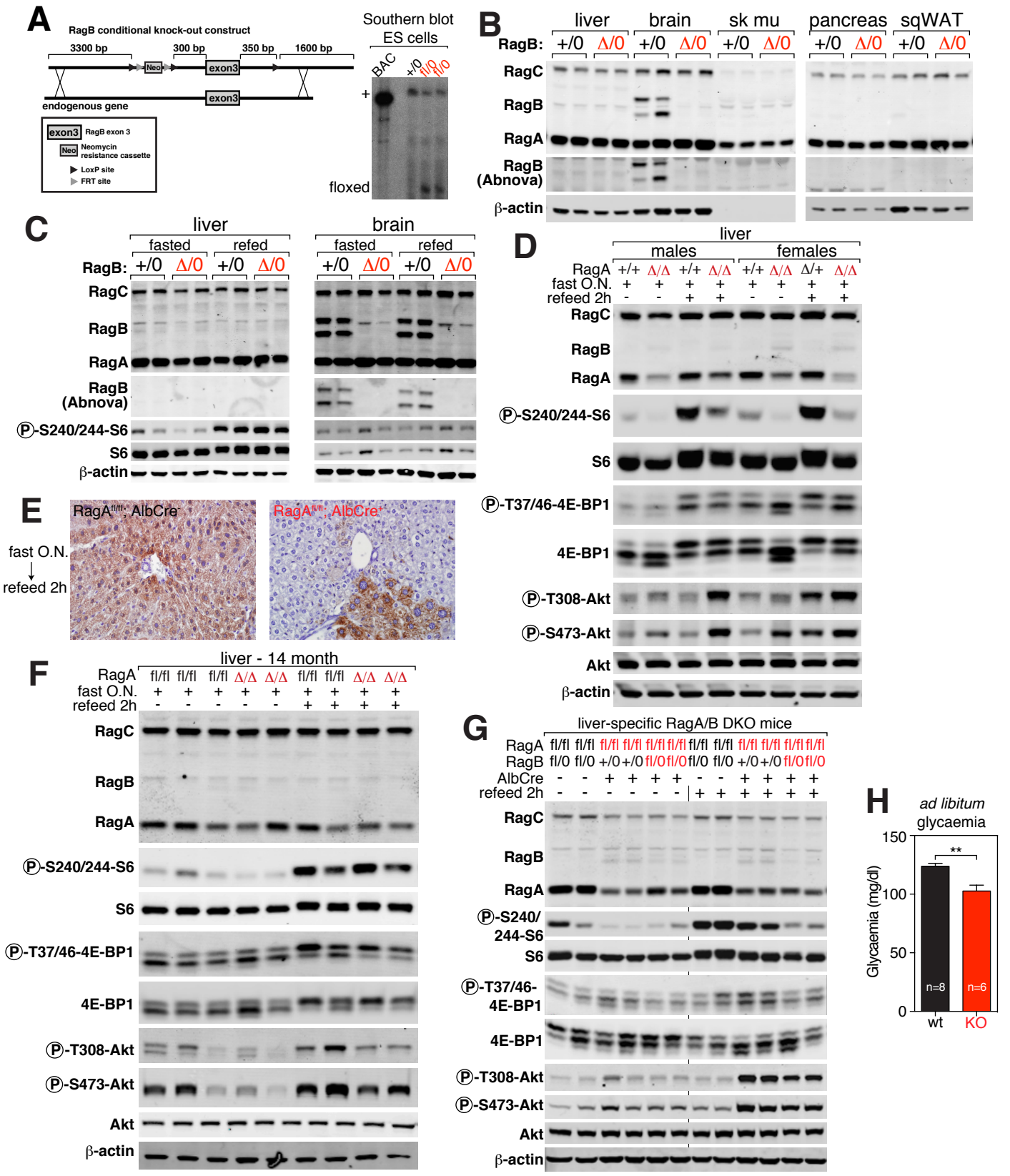
-Supplementary Experimental Procedures.



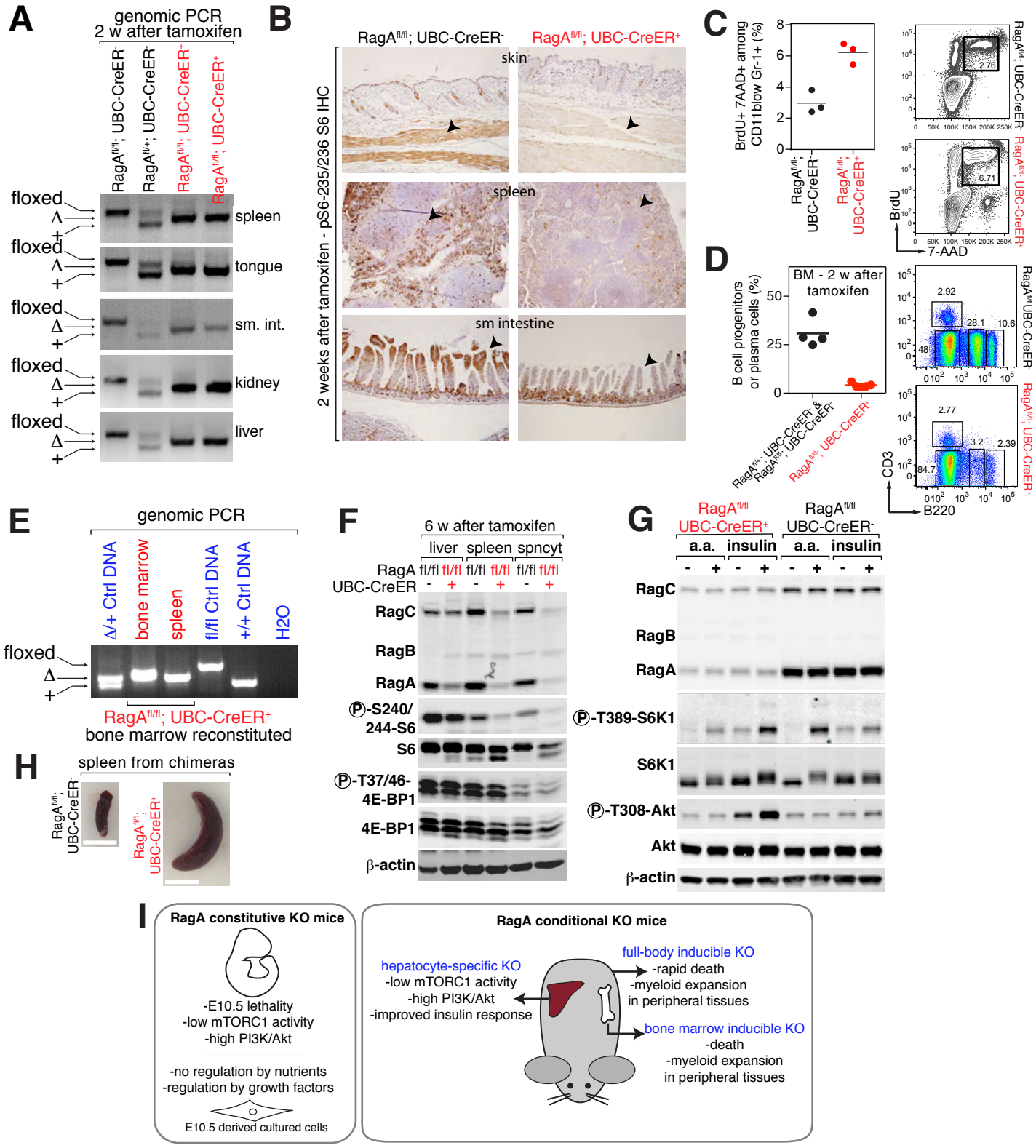
Supplementary Figure 1, relative to Figure 1. (A) Left: Strategy to generate RagA conditional knock-out mice. Right: Southern blot of genomic DNA from targeted ES cells cut with SacI. Fragments correspond to wild-type band 15 Kbp and the RagA floxed allele band 7.5 Kbp. (B) Additional RagA$^{-/-}$ to those on Figure 1a. (C) Whole-embryo protein extracts from RagA$^{+/+}$ RagA$^{+/-}$ and RagA$^{-/-}$ littermates were analyzed by immunoblotting for the indicated proteins (in addition to those in main Figure 1). Note the differential Akt activity in the RagA$^{-/-}$ reabsorbed versus non-reabsorbed embryo in the right panel. (D) Control samples from Figure 1c. RagA$^{fl/+}$ MEFs were infected with controls adenovirus (Ad-Ø) or adenovirus encoding the Cre recombinase (Ad-Cre) and then starved for amino acids for 1 h and, where indicated, re-stimulated for 10 min. Total protein extracts were then analyzed by immunoblotting for the indicated proteins. (E) RagA$^{fl/+}$ and RagA$^{fl/fl}$ MEFs were infected with control adenovirus (Ad-Ø) or adenovirus encoding the Cre recombinase (Ad-Cre) and then starved for amino acids or for glucose for 1 h and, where indicated, re-stimulated for 10 min. Total protein extracts were then analyzed by immunoblotting



Supplementary Figure 2, relative to Figure 2. (A) Absence of RagA protein and increased RagB protein, determined by immunoblotting, in RagA KO cell lines. (B) mRNA levels of mTORC1-related genes. RagA$^{-/-}$ cells lack RagA mRNA, show increased RagB mRNA, and normal expression of RagC and other mTORC1-related genes. n=3 per genotype; mean±SD (C) RagA$^{-/-}$ cells exhibit sensitivity to mTORC1 inhibition by rapamycin, as determined by immunoblotting for mTORC1 activation markers from whole cell extracts. (D) KD of the Rheb GTPase abrogates mTORC1 activity in RagA-deficient cells. (E) Immunofluorescence for the obligate RagA protein partner RagC and the lysosomal membrane marker Lamp2. RagA$^{-/-}$ cells show diffuse RagC staining that does not co-localize with Lamp2. (F) Lamp1 localization by immunofluorescence in methanol-fixed cells. RagA$^{+/-}$ and RagA$^{-/-}$ cells show similar pattern of staining, indicating a similar lysosomal morphology. (G) mTOR localization by immunofluorescence in cells stably expressing RagB protein. Expression of RagB in RagA$^{-/-}$ cells allows mTOR to be recruited to the lysosomal surface in the presence of amino acids. (H) Expression of RagB in RagA$^{-/-}$ cells partially suppresses the activation of Akt, as revealed by decreased phosphorylation at Thr308. (I) Expression of RagA or RagB in RagA-null cells leads to amino acid sensitive mTORC1 activity. (J) and (K) Regulation of mTORC1 activity and autophagy by prolonged amino acid starvation of cells. Cells were starved for amino acids for the indicated times and re-stimulated. MTORC1 targets S6K and ULK1, together with the autophagy marker p62 were determined by immunoblotting.



Supplementary Figure 3, relative to Figure 3. (A) Left: Strategy to generate RagB conditional knock-out mice. Right: Southern blot of genomic DNA from targeted ES cells cut with SacI. Fragments correspond to wild-type band (16 Kbp) and the RagB floxed allele band (6 Kbp). (B) Protein extracts of indicated tissues were immunobloted for RagA, B and C. (C) Protein extracts from liver and brain from overnight fasted and refed mice were obtained and mTORC1 activity was determined by immunoblotting. (D) Liver samples from cohorts of young RagA liver-specific KO male and female mice, fasted overnight and re-fed for 2 h, were extracted and levels of the indicated proteins were determined by immunoblotting. (E) Formalin-fixed livers from mice fasted and re-fed as in Figure 3b were processed for immunohistochemistry with anti-phospho-S235/236 S6. The morphology of groups of positive cells in the RagA$^{fl/fl}$; Alb-Cre+ mice is indicative of hepatocytes (F) As in (D), but samples were obtained from older mice (>1 year). (G) Mice with the indicated alleles of RagA & B were fasted and re-fed, as in Figure 3a, and liver protein extracts were immunoblotted for the indicated proteins. (H) Glycaemia was measured from *ad libitum* fed control (WT) and RagA$^{fl/fl}$; Alb-Cre+ (KO) mice.



Supplementary Figure 4, relative to Figure 4. (A) Genomic PCR from liver, spleen, kidney, tongue and small intestine (sm. int.) of the indicated genotypes 2 weeks after tamoxifen injections (B) Immunohistochemistry for phospho-S235/236 S6 in samples of the indicated genotypes 2 weeks after the start of tamoxifen injections. (C) Proliferation as determined by *in vivo* BrdU incorporation and 7-AAD staining in bone marrow samples of tamoxifen treated mice of the indicated genotypes on CD11b^{low} Gr-1^{+} CD115^{-} cells, and 2 representative examples of the FACS plots. (D) Bone-marrow derived cells were purified and B cell progenitors and plasma cells were quantified by B220 and CD-3 staining followed by FACS (mean and scatter plot, n=4 and n=5, respectively), and 2 representative examples of the FACS plots. (E) Genomic PCR as in (A), from samples from bone marrow reconstituted mice of the indicated genotypes (F) Whole protein from liver, spleen and purified splenocytes were extracted and immunoblotted for the indicated proteins. (G) Macrophages, after tamoxifen injections, were purified and cultured. Cells were then deprived of serum or amino acids, and re-stimulated with insulin or amino acids, and levels of RagA and mTORC1 activity were determined by immunoblots. (H) Representative spleens from bone marrow reconstituted mice with RagA$^{fl/fl}$; UBC-CreER- mice and RagA$^{fl/fl}$; UBC-CreER+ cells. (I) Schematic summary of the results presented herein.

Supplementary Table 1. Embryonic lethality of RagA$^{-/-}$ mice, related to Figure 1.

Crosses*	Time	RagA$^{+/+}$	RagA$^{+/-}$	RagA$^{-/-}$
RagA$^{+/-}$ x RagA$^{+/-}$	adult mice	51	121	0
	E13.5	5	6	0
	E11.5	7	13	5 (3^{s}+2^{r})#
	E10.5	26	60	31 (23^{s}+8^{r})

*: Parental mice were either RagA$^{STOP/+}$ or RagA$^{\Delta/+}$; results were similar an added to the present Table.

#: S indicates that the embryo was smaller, but still with identifiable embryonic structures and generally heart beating was detected. R indicates that the embryo was reabsorbed.

Table 2. Embryonic Lethality of RagB$^{-/-}$ mice, related to Figure 3.

Crosses	Time	RagA$^{+/+}$ RagB$^{-/0 \text{ or } -/-}$	RagA$^{+/-}$ RagB$^{-/0 \text{ or } -/-}$	RagA$^{-/-}$ RagB$^{-/0 \text{ or } -/-}$
RagA$^{+/-}$;RagB$^{-/0}$ x RagA$^{+/-}$;RagB$^{-/-}$	E10.5	9	8	3 (0^{s}+3^{r})

#: S indicates that the embryo was smaller, but still with identifiable embryonic structures and generally heart beating were detected. R indicates that the embryo was reabsorbed.

Supplementary Experimental Procedures

Generation of RagA and B knock out mice

All animal studies and procedures were approved by the MIT Institutional Animal Care and Use Committee. RagA locus was targeted by introducing LoxP sites and frt-neomycin-frt cassette flanking RagA exon. Homology arms were 1500 bp and 3000 bp for 5' and 3', respectively. LoxP sites were inserted 300 bp upstream and 500 bp downstream of the RagA exon. The frt-neomycin-frt cassette was inserted next to the 3' LoxP site (map of the targeting allele is shown in Supplementary Figure 1a). Similarly, RagB locus was targeted by introducing LoxP sites flanking exon 3 (map of the RagB targeting allele is shown in Supplementary Figure 3a). $RagA^{STOP}$ allele was previously described (Efeyan et al., 2013). Linearized constructs were electroporated into male v6.5 ES cells of mixed 129Sv/C57B6 background (v6.5). ES colonies were picked and identified by Southern blot and confirmed by PCR amplification of specific insertion products. Positive ES cells clones were then injected into blastocysts and transferred into pseudo-pregnant females to obtain chimeric mice. Pure C57B6 transgenic Cre strains of mice were then bred with RagA or RagB *floxed* mice.

Treatments of mice

Tamoxifen was dissolved in corn oil (Sigma) at 10 mg/ml and 200 μml per 25 g was injected i.p. for 7 consecutive days. For fasting/re-feeding experiments, fasting was performed from 6 pm to 9 am and then mice were injected with 30 % w/v glucose in PBS 100 μl per 30 g mouse, allow to feed *ad libitum*, or injected i.p. with 11.25 μl of insulin (HumulinR, Lilly) dissolved in 5 ml of PBS at a dose of 100 μl per 30 g mouse. For bone marrow reconstitution, host mice were lethally irradiated with 1200 rad divided in two

irradiation sessions 4 h apart, and purified $1x10^6$ bone marrow cells from either $RagA^{fl/fl}$; UBC-$CreER^+$ or $RagA^{fl/fl}$; UBC-$CreER^-$ were injected retro-orbitally 1 h after the last irradiation. For glucose tolerance test (GTT), and insulin tolerance test (ITT) mice were fasted overnight (GTT) or 6 h (ITT) and glycaemia was measured for 2 h after i.p. injection of glucose or insulin at the doses described above.

Preparation of MEFs

MEFs from E10.5 embryos were prepared by chemical digestion with trypsin, followed by serial passage when cells reached confluence. MEFs from E13.5 embryos were prepared by chemical digestion with trypsin for 15 min, followed by mechanical disaggregation.

Treatments of MEFs

For amino acids and glucose deprivation in MEFs, sub-confluent cells were rinsed twice and incubated in RPMI without amino acids and/or glucose, and supplemented with 10% dialyzed FBS, as described (Sancak et al., 2008). Stimulation with glucose (5 mM) or amino acids (concentration as in RPMI) was performed for 10 min. For serum withdrawal, cells were rinsed twice in serum-free DMEM and incubated in serum-free DMEM for the indicated times; 100 nM was used for insulin stimulation. Rapamycin was used at 10 nM. For acute deletion of RagA gene in early passage E13.5 MEFs, cells were transduced with adenovirus-encoded Cre, or empty adenovirus as control. For introducing RagB and RagA in RagA E10.5 MEFs, MEFs were infected with the pLJM1 lentivirus encoding for Metap2 (control protein), Flag-RagA or Flag-RagB and selected for stable integration.

Treatments of macrophages

Bone marrow-derived macrophages were isolated as described (Weischenfeldt and Porse, 2008). Briefly, bone marrow from femurs and tibias was plated on 10 cm bacterial grade petri dishes in macrophage media (RPMI containing 10% fetal bovine serum, penicillin and streptomycin, 2 mM glutamine and 30 % v/v L929-conditioned media). Media was replaced two days after isolation. Every two or three days, cells were passaged by scraping with a cell lifter or media was replaced. Five hundred thousand macrophages were seeded in 6-well tissue culture dishes and treated 48 h later. Cells were washed with PBS and incubated for one hour with RPMI lacking amino acids supplemented with dialyzed FBS or DMEM without serum and stimulated with amino acids or 100 nM insulin for 30 minutes.

Immunoblotting

Reagents were obtained from the following sources: anti phospho-T389 S6K1, phospho-S2240/244 S6, phospho-S235/236 S6, phospho-T37/T46 4E-BP1, phosho-T308 Akt, phospho-S473 Akt, phospho-S9 GSK3-b, phospho-T24/T32 FoxO1/3a, phospho-ERK1/2, phospho-ULK1; total Akt, S6, S6K1, 4E-BP1, GSK3-b, FoxO1/3a, RagA, RagC, ERK1/2, IRS1, p62, from Cell Signaling Technology (CST); anti RagB from Abnova; anti β-actin (clone AC-15) from Sigma. Cells were rinsed once with ice-cold PBS and lysed in ice-cold lysis buffer (50 mM HEPES [pH 7.4], 40 mM NaCl, 2 mM EDTA, 1.5 mM sodium orthovanadate, 50 mM NaF, 10 mM pyrophosphate, 10 mM glycerophosphate, and 1% Triton X-100, and one tablet of EDTA-free complete protease inhibitors [Roche] per 25 ml). Cell lysates were cleared by centrifugation at 13,000 rpm for 10 min. Protein extracts were denatured by the addition of sample buffer, boiled for 5 min, resolved by SDS-PAGE, and analyzed by immunoblotting.

Immunofluorescence and immunohistochemistry

MEFs were plated on fibronectin-coated glass coverslips at a sub-confluent density of 50-100,000 cells/coverslip. The following day, cells were transferred to amino acid-free RPMI, starved for 60 min or starved for 50 min and re-stimulated for 10 min with amino acids, rinsed with cold PBS once and fixed for 15 min with 4% paraformaldehyde, or with -20C methanol for 10 min. PFA-fixed coverslips were permeabilized with 0.05% Triton X-100 in PBS and then all coverslips were incubated with primary antibodies in 5 % normal donkey serum for 1 h, rinsed, and incubated with Alexa fluor-conjugated secondary antibodies (Invitrogen) diluted 1:400, for 45 min. Coverslips were mounted on glass slides using Vectashield (Vector Laboratories) and imaged on a spinning disk confocal system (Perkin Elmer) equipped with 405 nm, 488 nm and 561 nm laser lines, through a 63X objective.

Flow Cytometry

Total bone marrow and spleen cells were stained with the following conjugated monoclonal antibodies: CD3, CD19, B220, NK1.1, Ter119, CD11c, CD11b, Gr-1 (Ebioscience). Stained cells were analyzed on a LSR cytometer (BD Biosciences) and data analyzed on FloJo software (TreeStar). For BrdU incorporation, 1.5mg of BrdU was injected intraperitoneally into mice previously treated with tamoxifen 10-12 days before. After 6h, mice were sacrificed and BrdU incorporation was analyzed by flow cytometry by standard nuclear staining following manufacturer's instruction (BD Pharmingen).

Quantitative PCR

Total RNA was extracted with RNAeasy (Qiagen), retro-transcribed with Superscript III (Invitrogen) and used at 1:100 dilution in quantitative real time PCR in an Applied Biosystems thermocycler. 36B4 and β-actin were for normalization. The following primers were used:

RagA F	GAACCTGGTGCTGAACCTGT
RagA R	GATGGCTTCCAGACACGATT
RagB F	TTCGATTTCTGGGAAACCTG
RagB R	AGTTCACGGCTCTCCACATC
mTOR F	GGTGCTGACCGAAATGAGGG
mTOR R	TCTTGCCCTTGTGTTCTGCA
Raptor F	TGGCAGCCAAGGGCTCGGTA
Raptor R	GCAGCAGCTCGTGTGCCTCA
Pepck F	CGATGACATCGCCTGGATGA
Pepck R	TCTTGCCCTTGTGTTCTGCA
G-6-P F	GAAGGCCAAGAGATGGTGTGA
G-6-P R	TGCAGCTCTTGCGGTACATG
Glucokinase F	GAGATGGATGTGGTGGCAAT
Glucokinase R	ACCAGCTCCACATTCTGCAT
36B4 F	TAAAGACTGGAGACAAGGTG
36B4 R	GTGTACTCAGTCTCCACAGA
Actin F	GGCACCACACCTTCTACAATG
Actin R	GTGGTGGTGAAGCTGTAGCC

Statistical analyses

For Kaplan-Meier survival curves, comparisons were made with the Log-rank Mantel-Cox method. For qtPCR analyses, measurements of glycemia, and other comparison between pairs, non-parametric t-tests were performed.